Freshwater Quality Monitoring Protocol
San Francisco Area Network

Standard Operating Procedure (SOP) # 7

FIELD METHODS FOR SAMPLING NUTRIENTS

Version 1.01

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REVISION HISTORY LOG

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.0	8/5/05	M. Cooprider	Minor edits	Preparation for formal peer review	1.01

Only changes in this SOP will be logged. "Version numbers increase incrementally by hundredths (e.g. version 1.01, version 1.02, …etc) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 …). Record the previous version number, date of revision, author of the revision, identify paragraphs and pages where changes are made, and the reason for making the changes along with the new version number" (Peitz et al, 2002).

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ACKNOWLEDGEMENTS

SFAN staff borrowed from several other protocols and guidelines. Many thanks are extended to the authors of these documents, most notably:

Puckett, M. 2002. Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program ("SWAMP"). California Department of Fish and Game, Monterey, CA. Prepared for the State Water Resources Control Board, Sacramento, CA. 145 pages plus Appendices.

Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., eds., September 1999, Collection of water samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A4, accessed __*date*__ at http://pubs.water.usgs.gov/twri9A4/

O'Ney, S. 2004. Procedures for Collection of Regulatory Parameters, Version 1.0, Standard Operating Procedure #6. *In* Regulatory Water Quality Monitoring Protocol, Version 1.0, Appendix E-SOPs, National Park Service, Great Yellowstone Network. Bozeman, MT. 37 pp. plus appendices.

1.0 SCOPE AND APPLICATION

1.1 Nutrients and Their Relation to Water Quality

Nitrogen and phosphorus are the primary plant nutrients and are ubiquitous in the environment. Aquatic systems are either nitrogen limiting or phosphorus limiting in reference to algal growth. Algae use nitrogen and phosphorus at a ratio of about 7:1 by mass. A significantly more narrow ratio means that there is a greater supply of phosphorus than nitrogen and that nitrogen is limiting growth. In this case, nitrogen is referred to as the "limiting nutrient". In most Bay Area freshwater streams, nitrogen is the limiting nutrient (California Regional Water Quality Control Board, 2003).

If a system is nitrogen limiting, then inputs of nitrogen can cause excessive growth (eutrophication) or toxicity to aquatic organisms. Eutrophication occurs at lower levels than toxicity (California Regional Water Quality Control Board, 2003). Nitrate and un-ionized ammonia can cause nutrient toxicity. Excess nutrients can also harm aquatic life through depletion of oxygen. Excess algal growth leads to greater respiration by live algae and decomposition of dead plant and algal material. Oxygen producing photosynthesis only occurs during the day while respiration, an oxygen depleting process, occurs 24 hrs. Therefore, dissolved oxygen is lowest just before dawn.

Nitrogen occurs naturally in several forms in the environment. Organic nitrogen (N) is anything that is organically bound including protein and peptide nucleic acids, urea, and synthetic organic materials. Organic nitrogen varies from 100ug/L in lakes to more than 20mg/L in raw sewage. Organic nitrogen and ammonia together are referred to as Total Kjeldahl Nitrogen (TKN) named after the Kjeldahl method. Total Ammonia Nitrogen or TAN is chemically represented as NH_4^+ and is present naturally in surface waters and wastewaters. Un-ionized ammonia or UIA is chemically represented as NH_3 and is toxic to aquatic organisms at low levels. This is of particular concern in SFAN streams supporting threatened and endangered species including coho salmon and steeelhead trout, California red-legged frog, and the California freshwater shrimp. Total oxidized or inorganic N is nitrate and nitrite. Nitrate generally occurs in trace quantities in surface water (APHA/AWWA/WEF 1998). Anthropogenic forms of nitrogen include fertilizers and wastes from warm-blooded animals that contain ammonia and organic nitrogen (among other forms of nitrogen). These wastes can enter surface waters via surface runoff, groundwater flow, direct access of animals to a creek, leaky septic systems, and leaky sewer lines.

Phosphates also exists in different forms including: orthophosphate, metaphosphate (or polyphosphate) and organically bound phosphate. Orthophosphates are produced by natural processes and are found in wastewater. Polyphosphate forms are used for treating boiler waters and are found in detergents. Poly forms of phosphate can change to the ortho form in water. Organic phosphates are a natural part of the environment but may also result from the breakdown of organophosphate pesticides. The common mineral source of phosphorus is insoluble rock phosphate [Apatite - $Ca_5(PO_4)^3(F_3OH)$] (Swaddle, 1997 *In* Thompson and Chambers, 2000).

1.2 Nutrient Levels in SFAN Waters

The UC Berkeley report *A Review of Water Quality Monitoring Programs in the National Parks in Central Coastal California* (Stafford and Horne, 2004) contains additional background information related nutrients. Additional information about nutrient levels and sources in SFAN waters is included in the SFAN Preliminary Water Quality Status Report (Cooprider, 2004). Water quality standards for nutrients are listed in Section 1.0 of the Freshwater Quality Protocol Narrative.

The San Francisco Bay RWQCB has identified Tomales Bay and its tributaries, Lagunitas Creek and Walker Creek, as impaired by nutrients. Once the nutrient monitoring implementation plan is complete, SFAN staff will be collaborating with the RWQCB regarding monitoring of key nutrients, primarily nitrogen parameters, in Lagunitas Creek and its tributaries, including Olema Creek. A *Conceptual Approach for Developing NutrientTMDLs for San Francisco Bay Area Water Bodies* was prepared by the San Francisco Bay RWQCB (RWQCB, 2003).

1.3 Nutrient Sampling and Analysis Methods Overview

The San Francisco Bay RWQCB does not use depth-integrated sampling for nutrient TMDL monitoring (Peter Krottje, personal communication). Also, the RWQCB's Surface Water Ambient Monitoring Program (SWAMP) does not collect depth-integrated samples for bacteria. Regardless, in many cases with SFAN streams, there is not sufficient depth, except during storm events, to obtain a meaningful depth-integrated sample. In order to maintain consistency at all of the sites and throughout the sampling season, it is best to obtain a "grab" or "hand-dipped' sample.

Monthly monitoring will be conducted for nitrate and ammonia, and Total Kjeldahl nitrogen.

Table 1.0 Summary of methods for laboratory parameters (from Puckett, 2002)

Parameter	Sample Volume (mL)	*Method Detection Limit (MDL)	Preservation & Storage	Holding Time
Total Kjeldahl Nitrogen	600 mL	0. 2 mg/L	Unfiltered; Cool to < 4° C; dark	7 days (28 days max)
Ammonia	500 mL	0.05 mg/L	Unfiltered; H_2SO_4, preservative, Cool to < 4° C	48 hours; or 28 days with preservative
Nitrate	150 mL	0.05 mg/L	Unfiltered; Cool to < 4° C; dark	48 hours
Nitrite	150 mL	0.01 mg/L	Unfiltered; Cool to < 4° C; dark	48 hours

* There are often several approved methods and they vary depending on the lab and the type of instruments that they have. Also, methods change and improve over time. The important consideration is that the labs use a method that has the desired MDL. MDLs are those recommended by the San Francisco Bay Regional Water Quality Control Board (Peter Krottje, personal communication, 1 July 2005).

2.0 TECHNIQUES

Tips for collection of nutrient samples:

- Collect water samples first before disturbing the sediment
- Note potential sources of contamination at each site
- Wear appropriate disposable, powderless gloves
- Use correct sample-handling procedures to avoid sample contamination
- Establish a routine for sample collection; use a consistent sampling technique
- Obtain training for and practice field techniques under supervision before collecting water samples.
- Collect a sufficient number of appropriate types of quality-control samples
- Prevent nose, mouth, eye, and direct skin contact with water

Aseptic Technique (from O'Ney, 2004)

Disposable latex or rubber gloves should be used to collection of nutrient samples. Some individuals have severe allergic reactions to latex. Field staff must avoid touching the opening of the sample collection container or its cap, or having the sample touch hands or arms. For each sample:

- If collecting samples for regulatory purposes, wash and scrub hands thoroughly to the mid-forearm, using antibacterial hand soap (or a hand sanitizer at 50 ppm chlorine equivalency, if available).
- Open the sample container taking care to avoid touching the inside surfaces or otherwise causing contamination
- Remove a glove by holding it from the wrist side opening inner surface. Avoid any contact with the outer surface of the glove.
- Do not touch anything with the exterior of the glove except the sample.
- If you have concern that the glove may be contaminated, discard that glove and use another sterile glove.
- With the gloved hand, collect the sample.
- After sample has been collected, close the sample container, remove and discard the glove and

Sample Bottles

Use 150 to 600 mL plastic nutrient sample bottles (supplied by the laboratory). It is important to have extra bottles as they occasionally can be swept away in current or contaminated. Some laboratories provide bottles with relatively "waterproof" labels already attached. Other labels are more susceptible to wear and general consist of regular paper. If this is the case, it is best to place a scotch tape over the label. It is sometimes easier and more efficient to label the bottle before sampling; this avoids having to dry off the bottle or write on a wet label. Pre-labeling (in the office or field vehicle) can also save time in the field (important during rain events).

Laboratory-supplied bottles contain a small amount of sulfuric acid preservative for samples to be analyzed for ammonia. These bottles should be clearly marked with "H_2SO_4". Since this is a strong acid, avoid contact with skin. Use a clean "transfer" bottle to collect the sample, then transfer it to the bottle containing preservative. Bottles for analysis of other nitrogen parameters will not contain preservative.

Collecting the Samples
(Adapted from USGS-NFM #5)

Prepare for sampling

1. Upon arrival at the field site, set out safety equipment such as traffic cones and signs.
2. Take extra bottles in case of contamination or loss
3. Take enough bottles to obtain QA/QC samples (see QAPP)
4. Label bottles but leave "time" field blank until actual sample collection

Determine the sampling location

1. Visually inspect the stream from bank to bank and longitudinally, observing velocity, width, and depth distribution, and apparent distribution of sediment and aquatic biota along the cross section. Note and document the location of stagnant water, eddies, backwater, reverse flows, areas of faster than normal flow, and piers or other features along the cross section.
2. Check the site list to determine whether the sample is to be collected in a pool or flowing area (or both). If sampling from a flowing area, identify the area of the stream that appears to be completely mixed (the centroid of flow). This may be determined ahead of time from reliable discharge measurements (see Initial Site Establishment – SOP#11 and Flow Measurement – SOP#9). Do not disturb the sediment before collecting a water sample.
3. For pools, if shallow (< 1 ft) take measurements at a middle depth. If the pool is from 1-4 ft deep collect a sample at a depth that meets monitoring objectives (Wilde et al, 1999)
4. For flowing water, sub-surface samples are taken at 0.1 m (4 inches) below the water surface if water level is < 5 ft (1.5 m). Samples are collected at the surface when water depth is < 0.1 m (Puckett, 2002). Sampling from the shoreline of any water body (meaning standing on shore and sampling from there) is the least acceptable method, but in some cases is necessary (Puckett, 2002).
5. Collect the nutrient sample at the same location as you will be collecting the bacteria sampling and measuring core parameters.

Water samples are collected from a location in the stream where the stream visually appears to be completely mixed. Ideally this would be at the centroid of the flow (*Centroid* is defined as the midpoint of that portion of the stream width, which contains 50% of the total flow), but depth and flow etc. do not always allow centroid collection (Puckett, 2002).

Collect the sample

Note: Collect water samples first to avoid disturbing the sediment and re-suspending sediment or bacteria

The USGS uses the "Hand-dip" method (Myers, 2003) if stream depth or velocity is not sufficient to use depth-integrated sampling. The procedure minimizes the collection of surface films and avoids contact with the streambed. The method is as follows:

1. Open plastic bottle; grasp the bottle near the base, with hand and arm on the downstream side of the bottle.
2. Without rinsing, plunge the bottle opening downward below the water surface. Allow the bottle to fill with the opening pointed slightly upward into the current.
3. Inspect each sample, looking for overfilling and (or) the presence of large amounts of particulates that might have been captured due to excessive streambed disturbance during sample collection. If you note either or both of these conditions, discard the sample, making sure there are no residual particulates left in the container, and resample.
4. Place the sample bottle in an ice-chest immediately. [NOTE: Use blue ice (often provided by labs), not wet ice to avoid possible contamination by contact with the melt water.] Ensure that the bottle label is completed with the date, time, site ID, and initials of field personnel.
5. Check the temperature of the ice-chest and refrigerator (if used); it must be between 1-4 °C. Samples should be stored in the dark.
6. Ensure that the samples are transported to the laboratory within the 6 hour EPA hold time.

3.0 FIELD PREPARATIONS AND LABORATORY COORDINATION

When starting work with a new laboratory, the Water Quality Specialist should develop a good working relationship with a laboratory manager and also a chemist/microbiologist. Discuss analytical methods, detection limits, holding times. Obtain official chain of custody forms from the lab as well as any needed bottles, cooler, and ice packs if the laboratory provides these. Discuss sample drop-off and pick-up possibilities. Also discuss the labs' capacity for the number of samples you will have. General tasks list include:

- Notify the lab at the beginning of the season, or as early as possible, of your sampling schedule
- Call the lab the day before or the morning of sampling to verify sample collection
- If at all possible, schedule sampling early in the week rather than later.
- Fill out the chain of custody form ahead of time except for the sample time

The chain of custody form is a means of tracking samples from receipt in the laboratory through analysis, to final disposal of the sample. It should be filled out in ink. The chain-of-custody Forms travel with the samples during the transfer, and are filed in the laboratory project files. Upon arrival at the laboratory, the "sample custodian" at the lab inspects the sample containers to ensure that the sample seals are intact and the sample containers have not been damaged. If any seals have been broken and/or any sample containers damaged, the sample custodian records the condition of the seals and containers on the chain-of-custody Form. The sample custodian takes custody of the samples by signing, dating, and noting the time in the on the chain-of-custody form.

Once at the laboratory, if samples need to be subdivided and submitted to another laboratory sub-contractor, this information should be noted on the original chain-of-custody form, and a new chain-of-custody form with the other lab should be initiated (Puckett, 2002).

<u>Equipment Checklist</u>
Scotch tape
Hand sanitizer
Bottle labels
Disposable gloves
Sample bottles
Data sheets printed on waterproof paper
Ice chest
Thermometer for ice chest
Chain-of-custody form
Sharpie (permanent pen)
Water jug for washing hands
Soap

Also, consult SOP#2 for safety equipment.

4.0 QUALITY ASSURANCE/QUALITY CONTROL

Duplicate samples should account for 10% of the total number of nutrient samples collected. It is also recommended that a field blank and trip blank be conducted (1 for every 20 samples). The lab will conduct a "matrix spike." Also consult the laboratory and request information on their quality control as well. Data Acceptability Criteria for Analysis of Water Quality Samples and QA/QC requirements are discussed further in the QAPP.

5.0 DATA REPORTING

A data analysis overview is provided in the protocol narrative. Details of data analysis are discussed in SOP #10.

Regardless of the form of the nitrogen measured, it should be reported in units of milligrams of nitrogen per liter (mg-N/L), so that there is the same amount of nitrogen in 2mg-N/L of nitrate as in 2mg-N/L of ammonia. Labs don't always report nitrogen this way; sometimes it is reported as the nitrate-nitrate. If it were reported in mg/L of ammonia and mg/L of nitrate, it would be difficult to compare them, since one molecule of nitrate is much heavier than one molecule of ammonia.

To convert from mg/L of ammonia to mg-N/L of ammonia, use the ratio of the molecular weight of nitrogen to the molecular weight of ammonia (14:18). To convert from mg/L of nitrate to mg-N/L of nitrate, use the ratio of the molecular weight of nitrogen to the molecular weight of nitrate (14:62).

<u>Conversion factors:</u>
N-molecular weight = 14
Oxygen molecular weight = 16
N/NO_3 = 14/14 +48 = 14/62 = 0.2258 (approximate conversion factor)

So if you have 45 mg/L of NO_3 that equals 10 mg/L of NO_3-N
45 mg/L NO_3 * 14N/62 NO_3 = 10 mg/L of nitrate-N

NH_3:
N = 14
H = 1

N/NH_3 = 14/14 +3 = 14/17 = 0.8235 (conversion factor)

Calculating Unionized Ammonia

Ammonia results are often reported as total ammonia (TAN). The unionized ammonia (UIA), which is the toxic fraction, can be calculated as follows:

TAN x conversion factor from Table 1 = UIA (mg/L)

Using the stream and pH and temperature at the time of sample collection, determine the conversion factor from Table 1.

Table 2. Fraction of unionized ammonia in aqueous solution at different pH values and temperatures. Calculated from data in Emmerson et al. (1975).

	Temperature													
pH	42.0 (°F)	46.4	50.0	53.6	57.2	60.8	64.4	68.0	71.6	75.2	78.8	82.4	86.0	89.6
	6 (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310	.0369
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617	.7929
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351	.8585
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271	.9389

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